

This quick reference guide provides the basic instructions on startup, shutdown, experiment setup, and unmixing using SpectroFlo™ software. For detailed information, refer to the *Aurora User's Guide*.

■ Startup

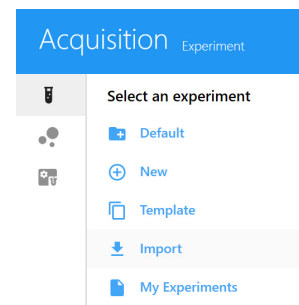
1. Check the sheath and waste tanks; fill and/or empty, if necessary.
2. Turn on the workstation, then turn on the cytometer.
3. Ensure that a tube of DI water is loaded on the SIP, then start SpectroFlo software and log in.
4. Wait for at least 30 minutes before running.

■ Shutdown

1. In the Cytometer menu from either the QC & Setup or Acquisition module, select **Fluidics Shutdown**.
2. Follow the prompts to load a tube with 3 mL of 10% bleach, followed by a tube with 3 mL of DI water.
3. Leave the tube of DI water on the SIP. Make sure the SIT is submerged in the water.
4. Turn off the cytometer.
5. Exit SpectroFlo software.

■ Data Acquisition

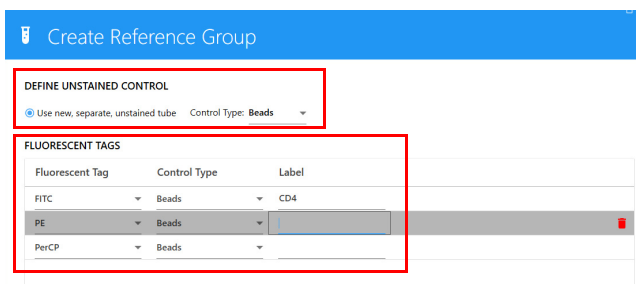
Click Acquisition from the Get started menu. The Acquisition Experiment menu allows you to open a new experiment, the default experiment, or a template. You can also import experiments, as well as access all your experiments, original and unmixed, from My Experiments.



Setting Up a New Experiment

A wizard guides you through the steps of creating a new experiment.

1. Click **New** in the Acquisition Experiments menu.
2. Enter an experiment name.
3. Select all the fluorescent tags used in the experiment. Tags are organized in default groups by laser.
4. Select **+** **Reference Group** to create a group for tubes that will be used as reference controls.
5. Select the control type (Cells or Beads) for the unstained control. The unstained control needs to be of the same type and prepared in the same way as the samples.



Then select the control type for each single-stained control (fluorescent tag).

You can also enter the labels associated with each fluorescent tag.

6. Click **Save**.

- Create groups for your samples and add tubes by selecting \oplus Group and/or Tube.
- Select labels for the sample tubes.
- Click **Next** when all tubes have been created and labeled.
- Select the worksheets for acquisition. Worksheets can apply to all tubes in the experiment, group, or individual tubes.
 - Select the Default Raw Worksheet (Raw) for the reference controls.
 - Select the desired (or Default) Unmixed Worksheet for the samples.
- Select the stopping gate, events to record, and stopping time.
- Click **Save and Open** to open the new experiment. The panes in the left column contain the controls used for acquisition.

Start/stop acquisition and record data.

Set the flow rate and events to display.
View real-time acquisition counters.

Select and save User Settings.

Select Gain, Threshold, Signal, or Lasers to adjust the corresponding instrument settings.

The screenshot shows two panels from the software interface. The top panel, 'Acquisition Control', includes a 'Tube_001' header, a control bar with 'Start', 'Record', 'Stop', and 'Restart' buttons, and a section for 'Flow Rate: Medium' and 'uL/Min: 27.67'. Below this are 'Event Rate: 0', 'Abort Rate: 0', 'Threshold Count: 0', 'Time Elapsed: 00:00:00 (HH:MM:SS)', and 'Events to Display: 10,000'. The bottom panel, 'Instrument Control', shows 'User Settings: Cytek Assay Setting' with 'Save' and 'Save As' buttons. It features a table for adjusting instrument settings:

GAIN		THRESHOLD		SIGNAL		LASERS	
FSC	SSC						
V1: 1,156	V2: 1,066	V3: 912	V4: 802	Violet Blue Red			
V5: 720	V6: 686	V7: 771	V8: 812				
V9: 912	V10: 914	V11: 747	V12: 643				
V13: 764	V14: 863	V15: 1,204	V16: 811				

- Click **Start** to view the data. **Start is enabled when a tube is loaded.** View all the controls and make any instrument adjustments necessary to ensure populations are on scale before you begin recording.
- Click **Record** when you are ready to begin acquisition.

To unmix, follow the appropriate workflow in the following section.
For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Unmixing

Raw vs Unmixed Data

Both *raw* and *unmixed* data are saved. Raw data, where no unmixing is performed, contains all fluorescence information for each detector. Unmixed data has been spectrally deconvolved to separate fluorochromes present on each particle based on a set of reference fluorochrome spectra. Data can be unmixed during or after acquisition, once all needed controls are acquired.

Unmixing Workflows (live unmixing is preferred.)

